THE ELECTRICAL CONDUCTANCE AND POTENTIAL ACROSS THE MEMBRANE OF SOME CELL NUCLEI

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The nuclear membrane of a variety of cells differs in at least two structural aspects from the cell membrane: it is double-layered and it presents annular figures 400 to 1,000 A in diameter which have a dense rim (1–10) and in cross-section sometimes appear as membrane discontinuities (2, 6). The physicochemical aspects of these differences have not yet been explored. Since the introduction of the microelectrode (11), the electrochemical properties of cell membranes have been extensively studied; however, those of the nuclear membrane, although of obvious importance in the mechanisms of exchange of materials between cytoplasm and nucleoplasm, have not yet been examined. The main reason for this neglect is the smallness of most nuclei which places them beyond the reach of direct electrical measurements. There are, however, a few animal cells, such as the salivary gland cells of *Drosophila* and a variety of oocytes, with nuclei large enough for studies with microelectrodes. For example, nuclei of large *Drosophila* gland cells measure 30 to 40 μ in diameter and of transparent frog and newt oocytes, 30 to 80 μ. This paper deals with some electrical properties of the nuclear membrane of such cells.

MATERIALS AND METHODS

Salivary glands of *Drosophila flavorepleta* (third instar larvae) were isolated and mounted in a bath of Schen's solution. Oocytes of *Triturus viridescens* and *Xenopus laevis* were isolated together with a piece of surrounding peritoneum and placed in Ringer's solution. Oocytes up to 300 μ in diameter and fresh gland cells are quite transparent. Their nucleus, its outline, and some of its internal details are clearly seen, without staining aids, under a darkfield or phase contrast microscope (Fig. 1).

Electrical measurements were done with microelectrodes, namely glass micropipettes of tip diameters below 0.5 μ, filled with 3 M potassium chloride, which had resistances of 10 to 35 megΩ and tip potentials below 2 mv. The nuclear membrane as well as the cell membrane seemed to seal well around such tips; there were usually no detectable changes in resting potential or current leakage over several minutes of insertion. There were also no observable signs of deterioration, such as are encountered on puncturing other types of nuclei with instruments of larger tips (12, 13). Electrode tip potentials (14) were measured in Schen's solution, cytoplasm, and nucleoplasm; they were found to be equal within 1 mv. For resistance (D.C.) measurements, a pair of electrodes was inserted in each cell or nucleus. One electrode served to pass square pulses of current of known intensity across the membrane, and the other one to record the resulting voltage drop and "resting" potential. A ground lead in the fluid around the cell was common for current delivery and potential measurements (see inset of Fig. 2 *C*). Since the nucleus is nearly spherical, the membrane resistance of unit area (transverse membrane resistance) could thus be determined with a high degree of accuracy.

All measurements were done on cell and nucleus material free of opacities within 30 minutes after isolation from the animal. Experiments on semi-isolated nuclei were done within 1 to 3 minutes after rupturing the cell membrane.

RESULTS AND DISCUSSION

Fig. 2 illustrates an experiment in which successively a gland cell and its nucleus are impaled with a microelectrode that measured potential with respect to the cell exterior. When the electrode is advanced from the cell exterior towards the nucleus, one finds, first, an abrupt change in potential, the cell membrane potential, as the electrode penetrates into the cell, and, then, another abrupt change of the same sign, as the electrode enters the nucleus (nucleus interior negative). The latter potential occurs clearly at the surface of the nucleus. It coincides with the crossing of the nucleus boundary by the electrode tip, and is usually preceded by a visible dimpling of

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the nuclear surface. It will hereafter be referred to as the nuclear membrane potential. No change of potential is seen as the electrode tip moves through the cytoplasm or nucleoplasm.

There is also a high electrical resistance associated with the nuclear surface. This is conveniently brought out by passing a train of current pulses of constant strength from the nuclear interior to the cell exterior with an electrode placed inside the nucleus, and by recording simultaneously and continuously the resulting voltage drops with a second electrode advanced progressively from the cell exterior to the nucleus. One observes then two sharp changes in resistance: one as the electrode penetrates the cell membrane, the cell membrane resistance, and another one as it enters the nucleus (Fig. 2 *B*). The latter coincides with the appearance of the nuclear membrane potential and is clearly associated with the nuclear surface; as the electrode moves through the cytoplasm or nucleoplasm, changes in resistance are several orders of magnitude lower than at the nuclear surface.

That the high resistance is confined to the nuclear surface is also shown by another kind of experiment. When the nuclear membrane is damaged by puncturing it in one or several spots with blunt micropipettes of, say, 2 μ tip diameter, or by strong electrical currents, the resistance drops immediately and the nuclear membrane potential declines gradually to zero. The resistance associated with the nuclear surface will here be referred to as the nuclear membrane resistance.

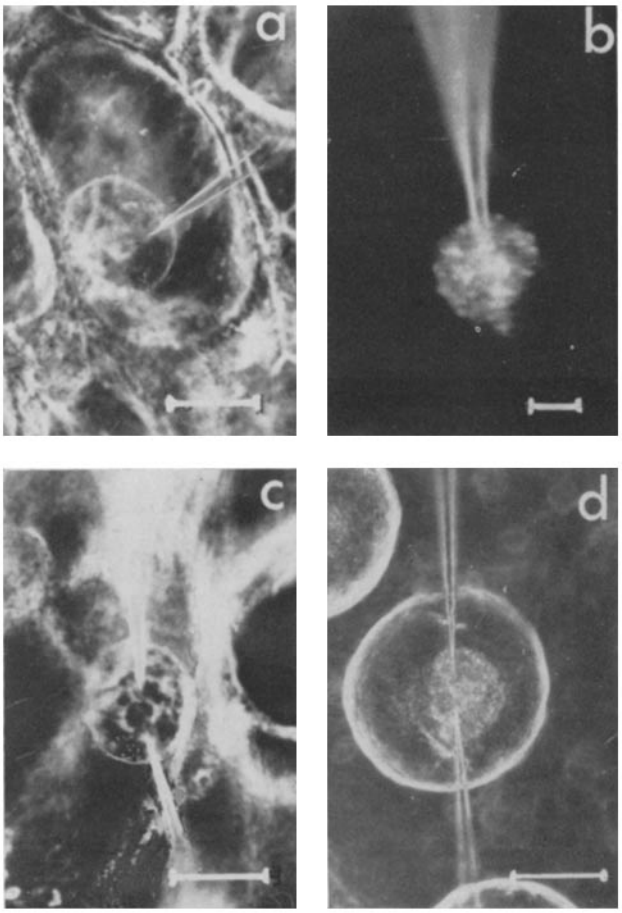

FIGURE 1

Darkfield photomicrographs of unstained nuclei impaled with one (*a*, *b*) or two microelectrodes (*c*, *d*). Nuclei of salivary gland cells *in situ* (*a*, *c*), and semiisolated (*b*) after destroying mechanically the cell membrane (cytoplasm that adheres to and surrounds the nucleus is not visible in this photomicrograph); and of frog oocyte (*d*). Calibration *a*, *b*, *c*: 25 μ; *d*: 100 μ.

Table I gives some typical resistance and potential values of cell and nuclear membranes. Nuclear membrane resistances were measured in each case under two conditions: first, in the nu-

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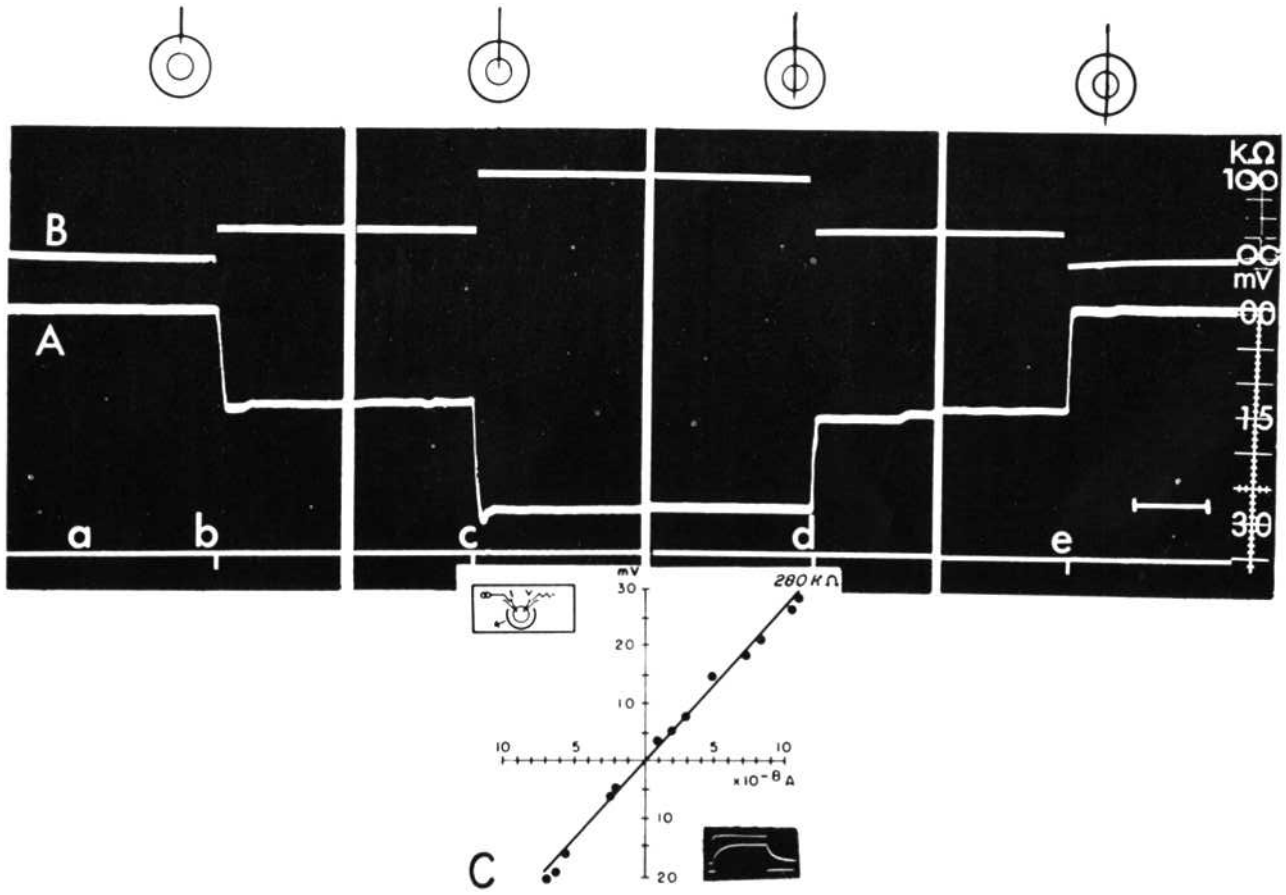

FIGURE 2

Potentials and resistance across cell and nuclear membranes (*Drosophila* gland cell). A microelectrode is advanced progressively in the direction cell exterior–nucleus. Beam *A* records continuously the potential (downward negative) as the electrode tip: *a*, is outside the cell; *b*, penetrates the cell membrane; *c*, enters the nucleus; *d*, leaves the nucleus and enters again the cytoplasm; *e*, reaches the cell exterior. Reference electrode is in the cell exterior. Time calibration: 0.05 sec., film interrupted for about 1 sec. in between photographs. *B*, a resistance plot on the same time scale. *C*, current voltage relation of the nuclear membrane after destruction of cell membrane. Abscissae: total nuclear membrane current passed with electrode *i* (inward current left; membrane area = 1.6×10^{-5} cm²). Ordinates: the resulting "steady state" voltage drop recorded with electrode *v* (hyperpolarization downwards). Lower inset, a sample record of membrane current and potential.

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TABLE I

Mean Resistance and Potential of Cell and Nuclear Membranes

Cell type	Cell membrane				Nuclear membrane			
	Potential		Resistance		Potential		Resistance	
	mv	Cases	Ωcm²	Cases	mv	Cases	Ωcm²	Cases
Gland cell (*Drosophila*)	12 ± 0.7	25	640 ± 14	9	13 ± 1.1	18	1.5 ± 0.4	8
Oocyte* (*Xenopus*)	11 ± 0.7	24	270 ± 84	9	0 ± 0.1	24	<0.001	19
Oocyte (*Triturus*)	15 ± 2.1	3	480 ± 132	3	0 ± 0.3	3	<0.001	3

Means of membrane potential and of transverse membrane resistance are given with their standard errors.
* Transparent oocytes of diameter between 80 and 300 μ only.

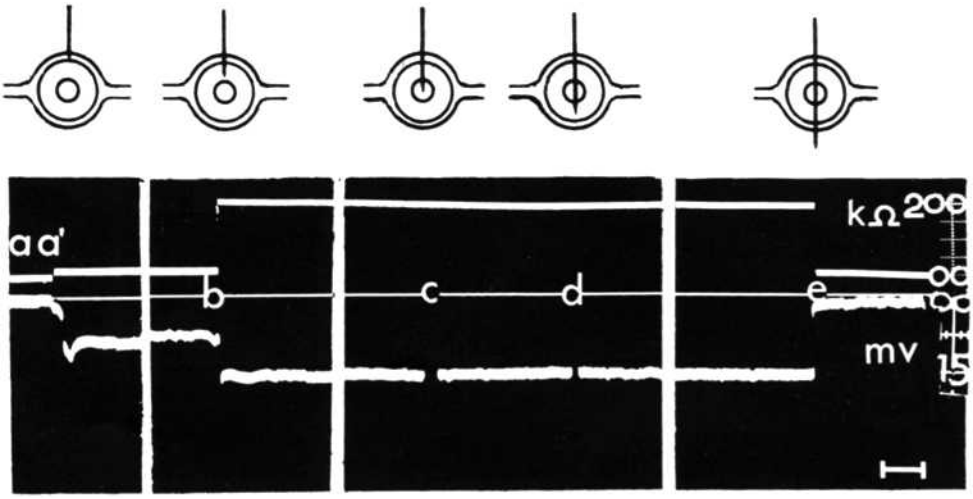

FIGURE 3

Potentials and resistance across cell and nuclear membrane of frog oocyte. Experimental sequence and labeling as in Fig. 2 except in *a'* when electrode penetrates through the outer epithelial layer of peritoneum around the oocyte. In *c* and *d*, beam is dimmed to signal electrode entering and leaving the nucleus surface. On its way out, the electrode stretches the elastic cell membrane and punctures it together with the epithelial layer. Note the potentials and resistances across the epithelial layer and cell membrane, and their absence across the nuclear membrane. Time calibration: 2 sec.

cleus *in situ*, surrounded by cytoplasm and cell membrane; and then, in the semiisolated nucleus, immediately after rupturing the cell membrane in one or two spots. In the first condition, the cell membrane, the resistance of which was previously measured alone, and the nuclear membrane were treated as resistances in series. In the second condition, the resistance of the cell membrane is completely abolished and that of the nuclear membrane can be measured directly. (The cytoplasm is viscous; most of it does not flow out through the ruptured cell membrane, but surrounds and keeps the nucleus in conditions of unchanged microscopic appearance and membrane potential.) There was in most cases fair agreement between the values obtained under the two conditions. Typically, the nuclear membrane shows no sign of excitation or rectification; its resistance is constant over a wide range of inward or outward currents (Fig. 2 *C*). There is, besides, a high capacitance associated with the nuclear membrane (15).

The finding of a high nuclear membrane resistance was surprising. The resistance is smaller than that of the cell membrane. It is also smaller than that of the cell membrane of a number of other cells (*cf.* references 16–20). It is, however, large enough to indicate that the nuclear mem-

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brane must be a formidable diffusion barrier even for ions as small as K^+, Na^+, or Cl^-. This is surprising in view of recent electron microscope studies which picture the nuclear membrane with large pore-like structures 400 to 1,000 A in diameter (2, 6, 9). From the present results it appears that these structures cannot provide a direct contact between nucleoplasm and cytoplasm. On the basis of a pore diameter of 500 A, a spacing of 1,000 A in between pore centers, and a total membrane thickness of 200 A, as given by Gay's electron micrographs for the nucleus of *Drosophila* salivary gland cells (7), and a resistivity of 50 Ω cm for nucleoplasm and cytoplasm, it is calculated that the nuclear surface exposed to the cytoplasm would be 15 to 23 per cent, and that such a perforated membrane would offer a resistance of the order of 10^{-3} Ω cm^2, three orders of magnitude below the observed one. It would seem, therefore, that the "pores," which have often been associated with the passage of large molecules, are either not freely communicating fenestrations in salivary gland cell nuclei, or are clogged with material of as high a resistivity as that of cell membranes. It is interesting in this connection that recent electron micrographs reveal the presence of material bridging the pore gap in some nuclei (4, 6, 21–24).

A high membrane resistance is, however, not a universal characteristic of nuclei. Oocyte nuclei were found to have membrane properties quite different from those of gland cell nuclei (Table I). In the two species of frogs and newts examined, the resistance of the oocyte nuclear membrane was so low that it was indistinguishable from that of the nucleoplasm or cytoplasm, and there was no detectable nuclear membrane potential (Fig. 3). The nucleus behaved merely like a small spheric droplet of nucleoplasm of low resistivity without the additional surface resistance of a membrane.

SUMMARY

Two kinds of nuclear membranes are described. One (of gland cells) has the high resistance of 1.5 Ω cm^2 and sustains a resting potential of about 13 mv between nucleoplasm (negative) and cytoplasm. Another one (of oocytes) has a resistance indistinguishable from nucleoplasm and cytoplasm and no resting potential. The former kind of nuclear membrane is a formidable diffusion barrier even for particles of ion size; the latter is more permeable.

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Dr. Kanno is on leave of absence from the Physiology Department of Tokyo Medical and Dental University, Japan.

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